Invertebrate life cycle responses to PAC exposure

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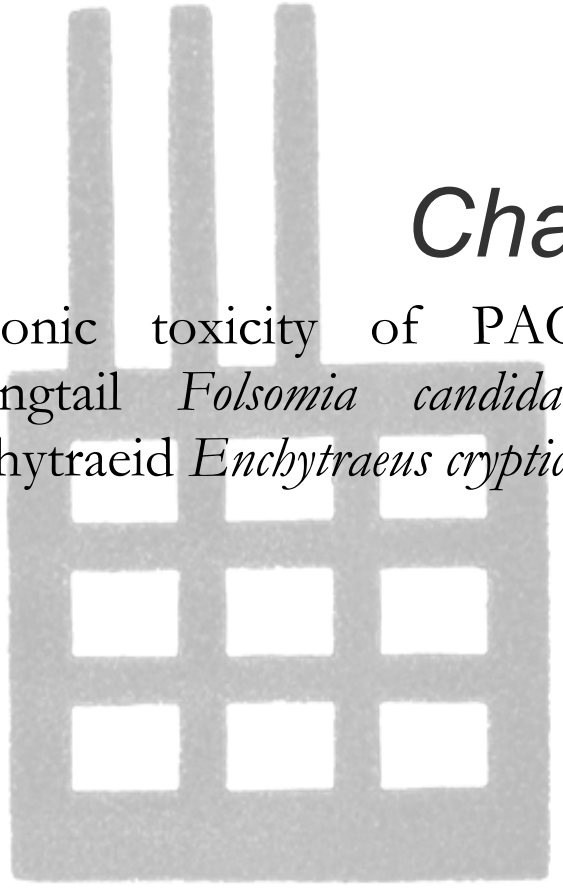

Chapter 2

Chronic toxicity of PACs to the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus*

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Abstract

An urgent need exists for incorporating heterocyclic compounds and (bio)transformation products in ecotoxicological test schemes and risk assessment of polycyclic aromatic compounds (PACs). The aim of the present study therefore was to determine the chronic effects of (heterocyclic) PACs on two terrestrial invertebrates, the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus*. The effects of 11 PACs were determined in chronic experiments using reproduction and survival as endpoints. The results demonstrated that as far as narcosis-induced mortality is concerned, effects of both homocyclic and heterocyclic PACs are well described by the relationship between estimated porewater 50% lethal concentrations and logK_{ow}. In contrast, specific effects on reproduction varied between species and between compounds as closely related as isomers, showing up as deviations from the relationship between porewater 50% effect concentrations and logK_{ow}. These unpredictable specific effects on reproduction force one to test the toxicity of these PACs to populations of soil invertebrates to obtain reliable effect concentrations for use in risk assessment of PACs.

Introduction

Contamination with Polycyclic Aromatic Compounds (PACs) often consists of a variety of different compounds, including hydroxylated, oxygenated, and chlorinated compounds as well as nitro- and amino-PACs. In addition, many heterocyclic compounds containing in-ring substitutions of nitrogen, sulfur, and/or oxygen atoms have been identified in such emissions (Nielsen et al., 1986), yet, current risk assessment for PACs focuses on homocyclic compounds only. Hence, an urgent need exists for incorporating heterocyclic PACs and (bio)transformation products (oxidation products) in ecotoxicological test schemes and risk assessment of PACs. Therefore, in addition to homocyclic compounds, the present study focused on azaarene analogues and stable azaarene metabolites. Azaarenes (i.e., PACs in which one carbon atom has been replaced by a nitrogen atom) are present in the environment in amounts up to 1 to 10% of those of their homocyclic analogues (Nielsen et al., 1999). Apart from their natural origin -for example, as alkaloids (Oshiro et al., 1998)- and the usual sources of PACs, basic azaarene structures occur as moieties of pharmaceuticals (Oshiro et al., 1998; Siim et al., 2000) and pesticides (Kuhn and Suflita, 1989; Crommetuijn et al., 2000). Special attention will be paid to isomerism, because it has been demonstrated that such slight differences in chemical structure can result in substantial differences in toxicity (Walton et al., 1983; Wood et al., 1983; Kumar et al., 1989; Kraak et al., 1997; Bleeker et al., 1998; Wiegman et al., 2001). Because soils and sediments are a major sink of PACs, the biota in these compartments should be protected by well-defined maximum permissible concentrations. Moreover, adequate understanding of the environmental risks of PACs is required, because bioremediation of polluted soils is expensive and the most affected sites need to be prioritized. Relevant toxicity data for soil organisms are scarce, though. In a recent review concerning the toxicity of PACs to terrestrial invertebrates, only 19 literature sources were mentioned (Achazi and Van Gestel, 2003). Comparisons between these few studies are complicated because of the wide variety of test conditions and experimental setups. The aim of the present study therefore was to determine the long-term effects of PACs on two terrestrial invertebrates belonging to different phyla, the springtail *Folsomia candida* and the enchytraeid (oligochaete) *Enchytraeus crypticus*. These two species presumably are exposed in different ways to PACs, because the springtail inhabits soil pores whereas the oligochaete perturbates the soil. Springtails also are in close contact with pore water through their ventral tube. Testing the parthenogenetic springtail *F. candida* allowed comparison with a closely related, sexually reproducing species, *Folsomia fimetaria*, for which toxicity data have become available (Sverdrup et al. 2001; 2002a;

2002b; 2002d). The effects of the PACs were determined in chronic experiments using reproduction and survival as endpoints.

Materials and methods

Test organisms

The parthenogenetic collembolan *F. candida* is a commonly used test species in soil toxicity tests (ISO, 1999). Recently, a standardized test method to evaluate effects of toxicants on enchytraeid survival and reproduction has been developed (OECD, 2000; Römbke and Moser, 2002). Both species are representatives of ecologically important functional groups and can reach high densities in various soil types (Wiles and Krogh, 1998; Didden and Römbke, 2001). They have relatively short life cycles, can be cultured easily in the laboratory, and are suitable for testing in both artificial and natural soils. Cultures of both species have been maintained at Vrije Universiteit for several years. The cultures were kept at 16°C under a 16:8-h light: dark photoperiod.

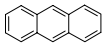
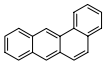
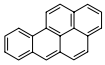
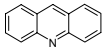
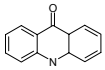
Test compounds

The selected PACs (Table 1) included six homocyclic compounds: naphthalene, the isomers phenanthrene and anthracene, benz(a)anthracene, pyrene, and benzo(a)pyrene. The chosen azaarenes quinoline and the isomers acridine and phenanthridine are analogues of the two- and three-ringed homocycles. The two azaarene metabolites 9(10*H*)-acridone and 6(5*H*)-phenanthridinone also are isomers. Several properties of these compounds are listed in Table 1. All compounds were purchased from Sigma-Aldrich (Steinheim, Germany) except for anthracene and benz(a)anthracene (Janssen Chimica, Beerse, Belgium).

Test soil

The toxicity tests were performed using a standardized natural soil (Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA 2.2, Speyer, Germany) sieved at 2 mm. This soil, characterized as a sandy loam (particle size distribution: 50-2000 μm, 75.3%; 2-50 μm, 16.6%; and <2 μm, 8.1%), was taken from a meadow that had been free of pesticide use or organic fertilization for more than four years. The total organic carbon content was 2.3% ± 0.2%, and soil pH (0.01 M $CaCl_2$) was 5.6 ± 0.4. Density, water-holding capacity, and cation-exchange capacity were 1.15 kg/dm^3, 46% (w/w), and 11 mval/100 g, respectively. The soil was kept at room temperature at a moisture content of 5% until used in the tests.

Table 1. Selected test compounds and some of their properties: Chemical Abstracts Service (CAS)-number, molecular weight (MW), purity, log K_{OW}, log K_{OC}, water solubility (Sw)

Compound	Structure	CAS No.	MW	Purit.	log K_{OW}	log K_{OC}	Sw (µM)
Homocyclic Polycyclic Aromatic Compounds (PACs)							
naphthalene		91-20-3	128.17	99+	3.47 [a]	2.7 [b]	249 [c]
anthracene		120-12-7	178.23	99+	4.53 [a]	4.3 [b]	0.37 [c]
phenanthrene		85-01-8	178.23	99.5	4.48 [a]	4.2 [b]	7.20 [c]
pyrene		129-00-0	202.26	98	4.84 [a]	5.0 [b]	0.72 [c]
benz[*a*]anthracene		56-55-3	228.29	99	5.54 [a]	5.1 [b]	0.048 [c]
benzo[*a*]pyrene		50-32-8	252.31	97	6.02 [a]	6.27 [d]	0.016 [e]
Heterocyclic PACs (azaarenes)							
quinoline		99-22-5	129.16	98	2.23 [a]	2.5 [b]	49000 [c]
acridine		260-94-6	179.22	97	3.27 [a]	4.1 [b]	260 [c]
phenanthridine		229-87-8	179.22	99+	3.44 [a]	3.8 [b]	25.7 [e]
Azaarene metabolites							
9(10*H*)-acridone		578-95-0	195.22	99	2.95 [d]		670 [e]
6(5*H*)-phenanthridinone		1015-89-0	195.22	97	2.70 [f]		1428 [e]

[a] Experimental values from Helweg et al. 1997; [b] Experimental values from Jonassen et al. 2003; [c] Values from Pearlman et al. 1984; [d] Experimental values from Thomsen 2002; [e] Calculated with *WSKOW*, Version 1.40. (Syracuse Research Corporation North Syracuse, NY, USA) ; [f] Value from Bleeker 1999.

Soil treatment

The soil was spiked with the following nominal concentration ranges of the selected compounds: For *F. candida*: naphthalene, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, and acridine at 62.5, 125, 250, 500, and 1000 mg/kg; quinoline and

phenanthridine at 31, 62.5, 125, 250, 500, and 1000 mg/kg; pyrene at 15, 31, 62.5, 125, 250, 500, and 1000 mg/kg; phenanthrene at 12.5, 25, 50, 100, and 200 mg/kg; and acridone and phenanthridinone at 10, 100, and 500 mg/kg; for *E. crypticus*: naphthalene, anthracene, phenanthrene, pyrene, benz[*a*]anthracene, benzo[*a*]pyrene, quinoline, and acridine at 62.5, 125, 250, 500, and 1000 mg/kg; phenanthridine at 31, 62.5, 125, 250, 500, and 1000 mg/kg; and acridone and phenanthridinone at 10, 100, and 500 mg/kg. Controls and solvent controls were included. Five replicates per concentration were used for the homocyclic compounds and the azaarenes, and four replicates per concentration were used for the transformation products. Acetone (purity, 99.8%; Riedel-de Haën, Seelze, Germany) was used as a carrier solvent, and equal volumes of acetone were added to all treatments. The PAC solution was added to a quarter of the soil sample, and the spiked soil was left overnight to allow the acetone to evaporate. The next day, the rest of the soil was added, water was added up to 46% ± 2% of the water-holding capacity, and the soil was homogeneously mixed. Soil PAC concentrations and pH were measured at the start of the experiment, after 10 d, and at the end of the experiment (28 d). To determine actual PAC concentrations and soil pH after 10 d, one additional replicate was prepared for the highest and second-lowest test concentrations, and one additional replicate was prepared for all concentrations to determine actual PAC concentrations and soil pH at the end of the test. The pH (KCl; average ± standard deviation) was 5.7 ± 0.37 at the start of the experiment and 5.3 ± 0.34 at the end of the experiment (28 d).

Experimental setup

Slight adaptations of the international guidelines for toxicity tests with *F. candida* (Wiles and Krogh, 1998; ISO, 1999) and *E. crypticus* (OECD, 2000) made it possible to test these organisms under closely comparable test conditions using the same series of PAC concentrations. The tests started with either *F. candida* that were synchronized to 11 to 13 d of age or adults of *E. crypticus* of approximately 0.4 to 0.6 cm that were adapted to clean LUFA 2.2 soil for 1 day. Each replicate consisted of 30 g (wet wt) spiked soil, containing 10 individuals, in 100 ml glass jars. These jars were closed off with black-plastic screw tops for the springtails and with perforated aluminum foil for the enchytraeids. All glassware used in the tests and in the PAC analysis, as well as the screw tops, were cleaned with 99.8% acetone and 96% methanol (both from Riedel-de Haën). At the start of the tests, 2 mg of food were added to each replicate on top of the soil (dried yeast grains for *F. candida* and crushed oatmeal for *E. crypticus*). To control fungal growth, extra food was added only when necessary, and loss of water through evaporation was compensated for by adding demineralized water once a week. Test containers were kept at 20°C and 80% humidity under a 16:8-h light:dark

photoperiod. The animals were extracted from the soil after the 28-d exposure period. The soils with *F. candida* were gently stirred with 100 ml of water, causing the surviving adults and the produced juveniles to float on the surface of the suspension. A digital photograph from this surface facilitated the counting of individuals with use of a colony counter (Bel-Arts Products, Pequannock, NJ, USA). Adult and produced juvenile enchytraeids were fixated by adding 5 ml of 96% ethanol to the soil. After a few seconds, the soil was rinsed out of the jar and into a plastic cup using 100 ml of tap water. Another 5 ml of 96% ethanol was added, and the suspension was gently stirred. A few drops of rose bengal dissolved in ethanol (1%) were added, and the cup, closed off with a lid, was shaken rigorously for 5 s. After leaving this suspension overnight at 4°C, the bright pink–colored enchytraeids were sieved over 160 μm and counted in a white, 80*50 cm photo tray. As a result of using adult, probably egg-bearing enchytraeid worms at the start of the test, the first-born juvenile worms reached the adult stage before the end of the 28-d exposure period. Therefore, adults with a body length of 8 mm or less were considered to be hatchlings of the original parents.

PAC analysis

Actual PAC concentrations were determined in single soil samples at the start of the experiment, after 10 d, and at the end of the experiment (28 d). From these samples, 5 g of moist soil were mixed with 5 g of anhydrous sodium sulfate (p.a. Merck, Darmstadt, Germany) and Soxhlet-extracted in hexane (purity, >97%; Biosolve, Valkenswaard, The Netherlands) or in acetonitrile (in case of the metabolites; analyzed high-performance liquid chromatography reagent; purity, >99.9%; J.T. Baker, Deventer, The Netherlands) for 5 h using 33*94 mm cellulose extraction thimbles (Schleicher & Schuell, Dassel, Germany). Cleanup of the hexane samples using Bakerbond spe™ Columns (Silica gel; J.T. Baker, Phillipsburg, NJ, USA) was performed in tests with anthracene and benzo(a)pyrene (both with *F. candida* and *E. crypticus*) and phenanthrene and benz(a)anthracene (both with *F. candida* only) but was found to be redundant for all other tests, because most PAC concentrations were sufficiently high. Moreover, azaarenes were not released from the column packing. Either directly from the Soxhlet flasks or after the cleanup procedure, 1.5 ml of the hexane extracts was added to 2 ml of acetonitrile (purity, >99.8%; J.T. Baker, Deventer, The Netherlands). The PAC was collected in the acetonitrile by blowing off the hexane using a gentle stream of nitrogen. These samples were then analyzed using a high-performance liquid chromatographic system consisting of a Vydac 201TP reverse-phase column (C18; length, 250 mm; inner diameter, 4.6 mm; film thickness, 5 μm) with a Vydac 201GD guard column (R-P

C18; length, 10 mm; inner diameter, 4.6 mm; film thickness, 10 μm) connected to a fluorescence detector (model FP-1520; Jasco, Essex, UK) and a UVD320s Ultraviolet diode-array detector (Gynkotek, Germering, Germany). When peaks were below detection levels, the hexane samples were concentrated up to 30-fold using Kuderna-Danish solvent evaporators. All volumes were checked by weighing on a 0.1-mg microbalance (R160P Sartorius, Göttingen, Germany), and samples were diluted with acetonitrile when necessary. In a separate experiment, PAC-spiked LUFA 2.2 soil samples were used to calculate extraction procedure recoveries and correction values for all tested PACs in the total extraction process.

Statistical analysis

The concentrations at which 50 or 10% mortality was observed (LC50 and LC10, respectively) and at which the reproductive output was reduced by 50 or 10% compared to the control (EC50 and EC10, respectively) were calculated according to the method described by Haanstra et al. (1985). Using SPSS® (Ver 10.0.5; SPSS, Chicago, IL, USA), the following logistic curve was fitted through the concentration–response plot using actual initial concentrations:

$$y = c/(1 + e^{b*(\log(x) - \log(a))}$$

where y is the effect parameter (survival or reproduction after 28 d), x is the exposure concentration (μmol/kg dry soil), a is the LC50 or the EC50 value, b is the maximal slope of the logistic curve, and c is the average survival or reproduction in the solvent control. For the LC50 and EC50 values, the corresponding porewater concentrations were calculated using the carbon content of the LUFA 2.2 soil and averages of experimentally determined (sorption to humic acids of different origin) organic carbon-water partitioning coefficients (*K*oc) as reported by Jonassen et al (2003) and by Thomsen (2002). Differences between effect concentrations were tested for significance at the 5% level by comparing the concentration-response plots with a generalized likelihood ratio test in Systat 5.2 (Systat, Evanston, IL, USA, 1992).

Results and discussion

PAC analysis

The extraction procedure applied in the present study resulted in recoveries between 80 and 105% for all tested compounds. Concentrations of anthracene, benz(a)anthracene, pyrene, benzo(a)pyrene, acridine, acridone, and phenanthridinone after 28 d were still at least 70% of the actual initial concentration. In contrast, naphthalene and quinoline concentrations at day 10 had dropped to 7 to 20% of the initial concentrations. All phenanthrene concentrations were still at least 70% of the

initial concentration at day 10, but in treatments with less than 1200 μmol/kg, the concentration dropped to 5 to 35% in the following period (recovery of treatments with 550, 842, and 1459 μmol/kg was 13, 34, and 96%, respectively, at the end of the enchytraeid test). A similar pattern was found for tests with phenanthridine (recovery in the 241, 629, and 2700 μmol/kg treatments was 30, 60, and 81%, respectively, at the end of the enchytraeid test).

Toxicity tests

Average control survival was greater than 80% for both test species. The average number of juveniles after 28 d in the control treatments was 683 ± 189 (n = 6) for *F. candida* and 983 ± 171 (n = 8) for *E. crypticus*. The spiking procedure with acetone had no effects on survival or reproduction of both test organisms. The solvent controls were used for further analysis. In the toxicity tests, the effects of the 11 selected compounds on the two endpoints of the two test species were determined. Figure 1 shows the concentration–response relationships for phenanthrene, its heterocyclic analogue phenanthridine, and its corresponding metabolite phenanthridinone. Both test species responded differently to the selected PACs, ranging from clear concentration-response relationships to no effects at the highest concentrations tested (Figure 1). The calculated LC10, LC50, EC10, and EC50 values for *F. candida* and *E. crypticus* are presented in Table 2. When two consecutive PAC concentrations resulted in no effect and a complete effect, respectively, no EC50 or LC50 value could be estimated, and the highest no-effect and the lowest total-effect concentrations are given instead. When the available concentration-response relationships were compared with a generalized likelihood ratio test, effect concentrations with overlapping 95% confidence limits in all cases did not differ significantly (generalized likelihood ratio test, $p < 0.05$), whereas nonoverlapping 95% confidence limits indicated a significant difference (generalized likelihood ratio test, $p < 0.05$).

Homocyclic PACs

The hydrophobic PACs benz[*a*]anthracene and benzo[*a*]pyrene as well as anthracene did not affect both soil invertebrates at the highest tested concentrations (Table 2). The relatively low maximum water solubility of hydrophobic PACs in soils probably is a limiting factor for the induction of effects. With increasing hydrophobicity, the water solubility of the PACs is decreasing more than the accumulation in the organism's lipids is increasing (Sverdrup et al. 2002b). Pearlman et

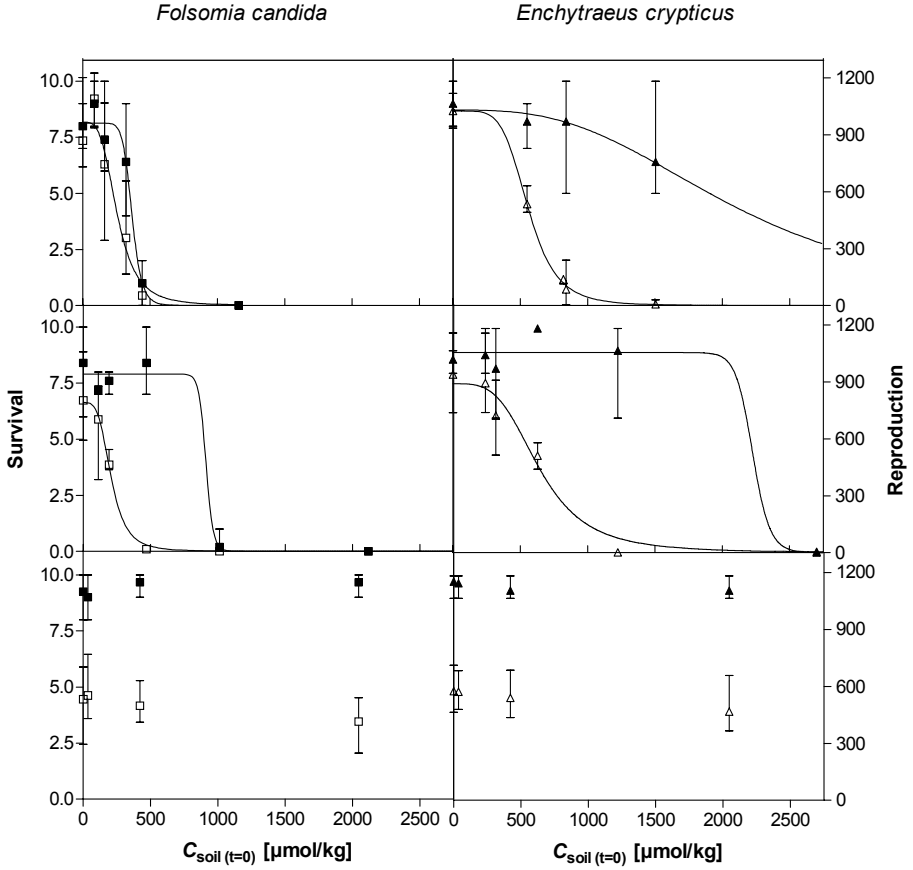


Figure 1. Concentration–response relationships for the effects of phenanthrene (PHE), phenanthridine (PHI), and phenanthridinone (PHO) on survival (black symbols) and reproduction (open symbols) of Folsomia candida (squares) and Enchytraeus crypticus (triangles) after 28 d of exposure in Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) 2.2 soil. Symbols represent the means, and error bars indicate minimum and maximum values. On the x-axis, the actual polycyclic aromatic compound concentrations in the soil at the start of the experiment are plotted. The curve-fitting procedure was performed as described by Mackay et al. (1999).

al. (1984) and Mackay et al. (1999) reported that the water solubility of anthracene is 18- to 20-fold lower than that of phenanthrene, which explains its lower toxicity. For all three compounds, it would serve no useful purpose to test higher concentrations, because it is nearly impossible to ensure a homogeneous PAC distribution in the soil because of the coagulation of the PAC while the acetone evaporates during the spiking process.

Heterocyclic PACs

Comparisons of the effects of naphthalene, anthracene, and phenanthrene and their azaarene-analogues quinoline, acridine, and phenanthridine clearly demonstrate the impact of the single N in-ring substitution on toxicity. However, no clear pattern could be observed in the changed toxicity: Anthracene was less toxic than acridine, most likely for the reasons discussed above. In contrast, naphthalene was more toxic than quinoline, which matches with its higher logK_{ow}. However, although phenanthrene has a higher logK_{ow} than its heterocyclic analogue phenanthridine, they were equally toxic. Hence, the observed differences in toxicity between the homocyclic PACs and their heterocyclic analogues are not well explained by logK_{ow}. Toxicity of PACs depends on their bioaccumulation from the pore water into the or-ganisms. The freely dissolved PAC concentrations in the pore water depend on the soil-pore water partitioning coefficients. These two parameters are not proportionally related to K_{ow}; therefore, porewater LC50 and EC50 values (µmol/l) were calculated using K_{oc} values from Table 1 and the organic carbon content of the LUFA 2.2 soil. The porewater LC50 and EC50 values were then again plotted against logK_{ow} (Figure 2). For both organisms, the inverse logtransformed LC50 values showed a positive relationship with the logK_{ow} of the test compounds. Bleeker et al. (2002) tested a series of PACs on larvae of the midge *Chironomus riparius* in a 96-h aquatic toxicity test. In Figure 2, the relationship between LC50 data and logK_{ow} from the acute aquatic toxicity tests by Bleeker et al. (2002) is presented as a gray line, with the corresponding 95% confidence limits as dotted lines. The plot shows that for those PACs that do exert toxicity in the present study, the effect concentrations for *F. candida* generally are well described by the relationship obtained for the midge *C. riparius*, whereas *E. crypticus* clearly is less sensitive than *F. candida* and *C. riparius* (see discussion below). Similar results were found by Sverdrup et al. (2001): soil-pore water EC10 values for the springtail *F. fimetaria* showed a fairly good correlation with no-observed-effect concentrations for *Daphnia magna*. This suggests a narcotic mode of action for the effects of both homocyclic PACs and azaarenes on survival of these widely differing invertebrates during chronic exposure.

Isomers and metabolites

The present study included three isomer pairs: anthracene and phenanthrene, the azaarenes acridine and phenanthridine, and the metabolites acridone and phenanthridone. The two metabolites did not affect both soil invertebrates at the highest tested concentrations, in contrast to their parent compounds acridine and phenanthridine. For these two azaarene isomers, a clear difference in effect concentration was observed, with phenanthridine being more toxic than acridine. This

difference disappeared, however, when the effects were expressed as porewater LC50 values (Figure 2). Anthracene did not affect both soil invertebrates at the highest tested concentrations, whereas for phenanthrene, clear concentration–response relationships were observed. This difference may be well explained by the low solubility of anthracene (see above); however, it is not neutralized by expressing effects as porewater LC50 values. Such strong, isomer-specific toxicities have been observed previously (Walton et al., 1983; Wood et al., 1983; Kumar et al., 1989; Kraak et al., 1997; Bleeker et al., 1998; Wiegman et al., 2001), and they clearly require refined molecular modeling to be explained.

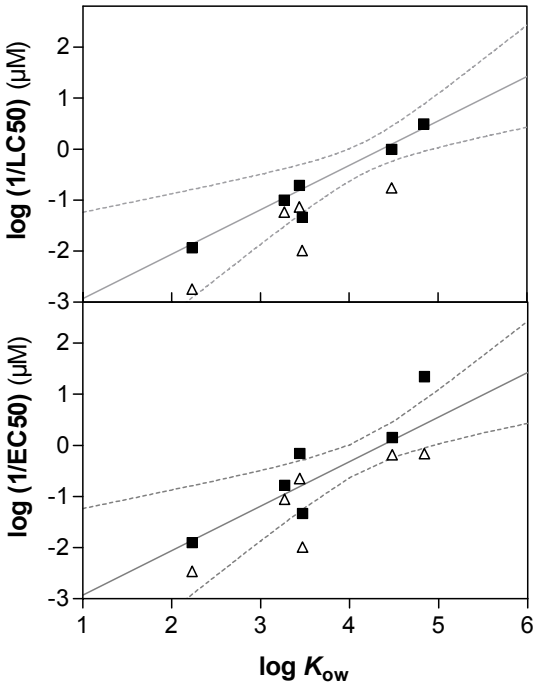


Figure 2. Calculated pore-water concentrations of polycyclic aromatic compounds (PACs) at 50% effect levels at the start of the 28-d soil toxicity tests. In the upper graph, the 50% lethal concentrations (LC50 values) for the polycyclic aromatic compounds are plotted against their K_{ow} values; the lower graph shows a plot of the 50% effect concentrations (EC50 values). Data for Folsomia candida are represented by squares, and data for Enchytraeus crypticus are represented by triangles. In both graphs, the solid line and the dotted lines represent the linear trend between 96-h LC50 data and K_{ow} and the 95% confidence intervals, respectively, obtained from water-only exposure of midge larvae to similar PACs (Bleeker et al., 2002).

Reproduction

Deviations from the relationship between effect concentrations and logK_{ow} values may indicate a specific mode of action. To detect if the selected PAC had a specific effect on reproduction, the graphs of the porewater LC50 and EC50 values plotted against logK_{ow} (Figure 2) were compared. Additional evidence was obtained by calculating LC50 to EC50 ratios for the PACs that exerted adverse effects (Table 3). The results indicate a specific effect of phenanthridine on the reproduction of both species, of phenanthrene on *E. crypticus*, and especially of pyrene on the parthenogenetic *F. candida*. Sverdrup et al. (2001) also found an effect of pyrene on the sexually reproducing *F. fimetaria*, demonstrating that the effect of pyrene is independent of the way the collembolans are reproducing. The specific effect of pyrene on springtail reproduction may have been caused by toxic metabolites. Hauser et al. (1997) found that the primary pyrene metabolite, 1-hydroxypyrene, was both acutely toxic in the Microtox™ test (Strategic Diagnostics, Newark, DE, USA; 50% inhibition of bioluminescence of the luminescent bacteria *Vibrio fischeri* at 0.68 mg/l) and genotoxic in the Mutatox test (inducing the ability to produce light in dark mutants of *V. fischeri* at 0.313 mg/l). For the isopod *Porcellio scaber* and the springtail *Orchesella cincta*, the primary metabolite 1-hydroxypyrene and the phase-2 metabolites pyrene-1-glucoside and pyrene-1-conjugate were measured rapidly after the start of oral exposure or exposure at contaminated field sites (Howsam and Van Straalen 2004; Stroomberg at al., 2004). The rate of PAC metabolism in isopods and springtails contrasts with that in earthworms, which metabolize PACs relatively slowly (Jager et al., 2002). Although no direct data are available regarding the biotransformation capacity of enchytraeids, toxic metabolites likely would not have been formed to the same extent as in the springtails. This could explain the finding that pyrene was much less toxic to the enchytraeids than to the springtails. A further agreement between the present study and that by Sverdrup et al. (Sverdrup et al., 2002a) is the effect of phenanthrene on enchytraeid reproduction, but remarkable differences also were observed: in the study by Sverdrup et al. (Sverdrup et al., 2002a), pyrene also affected enchytraeid reproduction, but this was not the case in the present study. It is concluded that specific effects on reproduction, which become evident during chronic exposure, are compound and species specific. The unpredictability of these effects questions the applicability of quantitative structure-activity relationships and acute to chronic ratios for predicting chronic sublethal effects of PACs.

Table 2. Polycyclic aromatic compound (PAC) concentrations in the soil at which 50% and 10% mortality (LC50, LC10) and 50% and 10% diminished reproduction (EC50, EC10, ± 95% confidence intervals) occurred for the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus* after 28 d of exposure in Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) 2.2 soil. Effect concentrations are based on measured initial concentrations in µmol/kg dry soil.

	Folsomia candida				***Enchytraeus crypticus***			
Compound	**LC50 (± 95% CI)**	**LC10 (± 95% CI)**	**EC50 (± 95% CI)**	**EC10 (± 95% CI)**	**LC50 (± 95% CI)**	**LC10 (± 95% CI)**	**EC50 (± 95% CI)**	**EC10 (± 95% CI)**
Homocyclic PACs								
naphthalene	88 – 409 [a]		88 – 409 [a]		220 – 2045 [a]		220 – 2045 [a]	
anthracene	>3814		> 3814		> 5038		> 5038	
phenanthrene	366	295	257	140	2109	997	559	370
	(337 – 394)	(268 – 322)	(201 – 313)	(86 – 193)	(1437–2780)	(362 – 1632)	(538 – 581)	(349 – 391)
Pyrene	741	377	104	56	> 4219		3359	
	(604 – 877)	(244 – 509)	(96 – 112)	(48 – 64)			(1864–4854)	
benz[*a*]anthracene	> 4345		> 4345		> 4070		> 4070	
benzo[*a*]pyrene	> 3690		> 3690		> 3690		> 3690	

Table 2 (continued)

Heterocyclic PACs (azaarenes)								
quinoline	628 (578–677)	541 (493 – 589)	581 (534 – 628)	469 (424 – 514)	4073 (1918–6228)	3084 (1047–5120)	2107 (1834–2381)	1402 (1143–1661)
acridine	1766–3991 [a]		1745 (1485-2005)	973 (724-1221)	4789 [b]		1518 – 5046 [a]	
phenanthridine	470–1012 [a]		208 (180 – 236)	116 (89 – 142)	1225 – 2701 [a]		646 (559 – 733)	355 (269–440)
Azaarenes metabolites								
9(10*H*)-acridone	>2242		>2242		>2242		>2242	
6(5*H*)-phenanthridinone	> 2046		> 2046		> 2046		> 2046	

[a] When two consecutive PAC concentrations resulted in no effect and a complete effect respectively, no EC50 or LC50 could be estimated, and the highest no effect and the lowest total effect concentrations are given instead.
[b] No reliable effect concentration could be calculated, but data suggest that this test concentration is very close to the LC50 (survival varies between 0 and 80%).

Table 3. Ratio between the 50% lethal concentrations (LC50 values) and the 50% effect concentrations (EC50 values) of polycyclic aromatic compounds (PACs) in Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) 2.2. soil for *Folsomia candida* and *Enchytraeus crypticus*. Values > 3, suggesting a specific effect on reproduction, are given in italic.

	Folsomia candida	***Enchytraeus crypticus***
Compound	**LC50/EC50**	**LC50/EC50**
	Homocyclic PACs	
naphthalene	1.0	1.0
phenanthrene	1.4	*3.8*
pyrene	*7.1*	1.3
	Heterocyclic PACs (azaarenes)	
quinoline	1.1	1.9
acridine	1.6	1.5
phenanthridine	*3.6*	*3.0*

Species-specific sensitivities

For all homocyclic PACs and azaarenes that did affect reproduction and survival at a certain level, *F. candida* appeared to be more sensitive than *E. crypticus*. Remarkable differences are the 32-fold lower EC50 value for springtails after pyrene exposure in soil and the 6.5-fold lower LC50 value after quinoline exposure (Table 2). Pyrene, phenanthrene, and acridine had been tested previously on a related collembolan (*F. fimetaria*) and the same enchytraeid (Sverdrup et al., 2001; 2002a; 2002b), and also in those studies, the collembolan was more sensitive than the enchytraeid. For *F. fimetaria*, a larger data set is available that shares six compounds with the present study. Generally, the results of both studies are in agreement, with benz[*a*]anthracene and benzo[*a*]pyrene not being toxic at the highest test concentrations and acridine being less toxic than the homocyclic PACs. Yet, a very distinct difference for anthracene was observed: in the present study, anthracene was not toxic at all, but it was more than six fold more toxic to *F. fimetaria* than predicted from its logK_{ow} (Sverdrup et al., 2002c). Comparisons between the few studies of terrestrial invertebrates are complicated because of the wide variety of test conditions and experimental setups. Still, some general trends may be derived: Sverdrup et al. (2002d) demonstrated that for the majority of PACs (eight compounds tested, sharing three with the present study), springtails were more sensitive than oligochaetes (earthworms and enchytraeids). The lower sensitivity of enchytraeids compared to springtails also was

observed in the present study. The present *F. candida* EC50 value for phenanthrene obtained in a soil with 3.9% organic matter is in the same range as that for *F. fimetaria* in soil with 2.8% organic matter (Sverdrup et al. 2001), but this value is much lower than the effect concentrations reported in soils with 10% organic matter (Bowner et al., 1992; Croau et al., 1999), most likely because of a lower availability in the soils with the higher organic matter content. Both Sverdrup et al. (2001; 2002a; 2002b; 2002d) and the present study found no effects of PACs with a logK_{ow} greater than 5.2. In contrast, Bauer and Pohl (Bauer and Pohl, 1998) reported that reproduction of *F. candida* already was affected at a benzo[*a*]pyrene concentration of 10 mg/kg, and effects of benzo[*a*]pyrene have been described for enchytraeids (Achazi et al., 1995) and the isopod *P. scaber* (Van Brummelen et al., 1996) as well. It is concluded that although some generalizations can be made, widely differing effect concentrations also have been reported, which can be explained only in part by differences in experimental setups.

Conclusion

The results of the present study demonstrate that as far as narcosis-induced mortality is concerned, effects of both homocyclic and heterocyclic PACs are well described by the relationship between porewater LC50 values and logK_{ow}. In contrast, specific effects on reproduction varied between species and between compounds as closely related as isomers. These unpredictable specific effects on reproduction showed up as deviations from the relationship between porewater EC50 values and logK_{ow}, and they force one to test the toxicity of these PACs to populations of soil invertebrates to obtain reliable effect concentrations for use in risk assessment of PACs. The long-term consequences of exposure to PACs in the field, however, should be analyzed in multi-generation experiments.

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